Negligible Sample Heating from Synchrotron Infrared Beam

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Infrared (IR) spectroscopy is one of the most sensitive analytical techniques for biological systems. Many common biomolecules, such as nucleic acids, proteins, and lipids have characteristic and well-defined IR-active vibrational modes.[1,2] Combining IR spectroscopy with microscopy yields a powerful tool for non-destructively probing biomolecules on a small size scale.[3] Conventional IR sources (thermal emission sources typically at a temperature of ~1200K) do not have sufficient brightness to operate with adequate signal-to-noise at spatial resolutions below ~50 μm.[4] However, with the recent use of synchrotron radiation (SR) light as an IR source[5-8] one can now obtain diffraction-limited spot sizes with high signal intensity in an infrared microscope. Several researchers have begun using these synchrotron infrared spectromicroscopy facilities for examining biological samples such as individual cells,[9-13] tissue samples,[4] and microorganisms in environmental settings.[14,15]

The high-brightness SR IR source (2 to 3 orders of magnitude brighter[6,8] than a conventional thermal IR source) is a great advantage when dealing with very small samples, such as individual living cells. Exposure to this more intense IR source has not been observed to cause any deleterious effects on living samples.[9,12,13] Since infrared light is too low in energy to be ionizing or bond breaking, the only possible effects on samples would be due to local heating. The power levels in the mid-IR spectral region of the SR beam are generally fairly low (~4 mW calculated integrated power), however experimentally determining if sample-heating effects from the synchrotron beam are indeed minimal is still required to prove that the technique is truly non-destructive. Here we present such a study and conclude that sample heating is, in fact, negligible.

When dispersed in water, the phospholipid dipalmitoylphosphatidylcholine (DPPC) forms bilayers that exist in at least two different states, depending on the temperature. These states are separated by a phase transition temperature (T_m) at around 315 K, when the bilayers are converted from a gel into a liquid-crystalline state.[16,17]

Infrared measurements were made on Beamline 1.4.3 at the Advanced Light Source.[7,8,18] Either a conventional thermal Globar™ source or the synchrotron source was used as detailed below. Mid-IR spectra were obtained at 4 cm^{-1} resolution with a Nicolet Instruments Magna 760 FTIR bench connected to a Nic-Plan IR microscope. A Ge-coated KBr beamsplitter and liquid nitrogen cooled MCT-A detector were used and data was collected in reflection geometry covering the 800-6000 cm^{-1} spectral region. Samples were mounted on an MMR Technologies optical transmission micro miniature refrigerator/heater fitted with KRS-5 windows for IR transparency. The sample temperature was set and monitored by an MMR K-20 programmable temperature controller to within an accuracy of 0.1 K.

A 20 :l dilution of the hydrated DPPC dispersion was placed on a gold-coated piece of glass, and a CaF_2 window was used to cover the sample creating a uniform layer thickness. Vacuum grease was carefully applied to the edges of the CaF_2 window to ensure the sample was sealed inside and no water loss during heating would occur. This sample was then mounted onto the temperature finger of the MMR heater/cooler with thermal grease. Measured reflectance spectra were ratioed to a background spectrum obtained with the same CaF_2 window on gold but without the DPPC present.

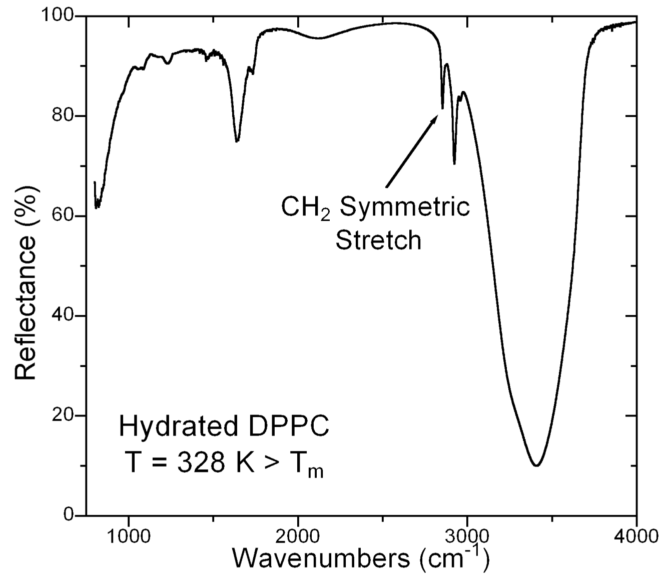

Fig. 1. Infrared reflectance spectrum of hydrated DPPC at T = 328 K. The largest absorptions are due to water, and the CH_2 symmetric stretch vibrational mode around 2850 cm^{-1} used as a temperature probe in this study is labeled.

Figure 1 shows the measured infrared spectrum of the hydrated DPPC at T=328 K (above T_m). As has been shown before,[16,17] the methylene (CH_2) symmetric stretch vibration at around 2850 cm^{-1} and the phosphate asymmetric stretching mode around 1240 cm^{-1} shift when the sample passes through the phase transition. For this study we monitored the CH_2 symmetric stretch mode (labeled in Fig. 1).

The CH_2 symmetric stretch mode's center frequency was measured using the less bright thermal Globar™ IR source as the sample temperature was increased and decreased through T_m. The results are displayed in Figure 2 along with a best fit to the data using a Boltzman expression with a linear background. The model function used for fitting matches the measured data excellently with an R^2 factor of 0.9976. The center of the transition was determined by this fit to be $T_m = 316.7 \pm 0.1$ K. The reproducibility of the data when cycling up and down in temperature indicates that the sample was well sealed and that the level of hydration of the DPPC remained constant at all temperatures.

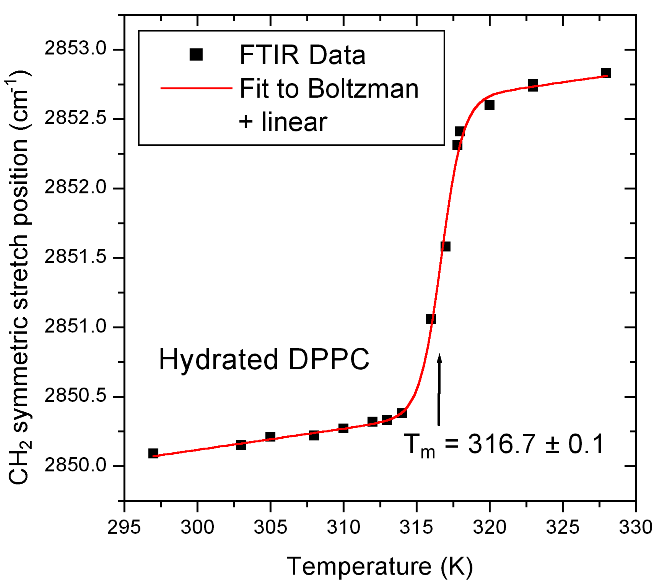

Fig. 2. Plot of the measured CH_2 symmetric stretch mode center frequency as a function of temperature. Data was obtained as the temperature was raised and lowered. The solid line is a best fit to the data using a Boltzman function with a linear background.

To test if the more concentrated flux of the synchrotron source causes any local sample heating, we set the sample temperature to be continuously held at 317.0 K. This temperature setting is where we would have maximum sensitivity to temperature changes as it is the point of greatest slope in Fig. 2. The IR source was then switched to the brighter synchrotron source and infrared spectra were acquired every minute for 30 minutes to look for any heating that occurred instantaneously or more slowly over time. The temperature of the sample within the beam spot was measured by monitoring the position of the CH_2 symmetric stretch mode as previously calibrated in Figure 2. The sample temperature fluctuated slightly over the 30 minutes, however no discernable longer-term trends were observed.

Statistical analysis of the measured temperature fluctuations determined that the average temperature rise due to the SR beam is a very modest 0.5 ± 0.2 K. We therefore conclude that the synchrotron IR source does not appreciably heat the sample under investigation. Since the test sample is mainly water, 98% by weight, we are confident that our result generalizes to most biological samples. For example, tissue[19] has a thermal conductivity of 1.43 mcal/sec/cm^2/°C/cm and bone[20] has a thermal conductivity of 1.40 mcal/sec/cm^2/°C/cm. Both values are essentially the same as the thermal conductivity of water[21] at 17 °C -- 1.42 mcal/sec/cm^2/°C/cm. Thus, the term non-destructive is truly appropriate for SR-FTIR spectromicroscopy.

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